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DEPARTMENT OF BOTANY AND BACTERIOLOGY

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Dr. Joshua Lederberg
The University of Wisconsin
College of Agriculture
Department of Genetics
Madison 6, Wisconsin

Dear Dr. Lederberg:

I have instituted a search for the copy of the M.A. thesis material in your letter of May 20 and have not as yet been able to find my copy. I suspect someone has borrowed it but it should show up. I find that the most interesting experiments were done after it was written and here is the essence of the thing.

Micrococcus aureus strains resistant to penicillin were grown together with similar cultures resistant to streptomycin. The cultures were also grown separately. After 5 or 6 hours incubation the cultures were plated to determine total numbers and also the proportion of each mutant present and the number that were resistant to both antibiotics. A typical result was:

	No. of organisms per cc from plate counts.		
	Penicillin mutant	Streptomycin mutant	Mixed culture
Nutrient agar	60,000,000	80,000,000	80,000,000
Nutrient agar + penicillin	48,000,000	28	22,000,000
Nutrient agar + streptomycin	210	60,000,000	30,000,000
Nutrient agar + both	100	12	4,000

The irradiation experiments involving the *E. coli* recombinations will appear in the next issue of the Proc. Nat. Acad. Sci. I believe that we discussed the details. I am afraid my lack of a good genetic background prevents me from seeing how a recombination of lethal factors could be involved in the haploid except by some very tricky mechanism for which there is as yet, no evidence.

I enclose a rough draft of a talk I gave about a year ago on E. coli transmutation. The abstract of this is published in J. Bact. We wasted some time on patent possibilities of the practical aspects of this work and the status of the problem is about where it was a year ago. The new students working on the problem have made little progress and are now attempting to isolate new strains for use in this work.

Kindest personal regards

Orville Wyss

Orville Wyss

OW/pam

Encl.

Many antibacterial agents are unsuitable inhibitors because of the resistant individuals which occur as spontaneous mutations in the microbial population. These individuals grow in the presence of the inhibitor and establish a resistant culture against which the inhibitor is ineffectual. We have devoted considerable study to the occurrence of such individuals. The recent work on directed mutations suggested that it may be possible to modify the resistance of a bacterial population by use of such techniques. The work at the Rockefeller Institute on the transformation of the pneumococcus and the studies of Bowin and collaborators (compte. rend. soc. biol. __139__, 1047, 1945) have shown that certain characteristics of a bacterial population can be modified by the use of a thymonucleic acid extract of another strain. If this method of inducing mutations is a general one it might be put to practical use in the field of bacterial resistance to inhibitory agents.

These studies were made with our laboratory strain of __E. coli__ (designated as the sensitive strain) and a sulfonamide resistant strain developed from it. A quantity of cells of both strains was harvested and extracts from each were prepared. The final procedure adopted is reported in table 4, but the early studies were made with the crude extracts and with the nucleoprotein rather than with the purified nucleic acids. These materials were sterilized by permitting them to stand under 70% ethanol overnight. They were then removed from the alcohol, dissolved in sterile buffer, and added to a series of test tubes which contained a synthetic __E. coli__ medium and several concentrations of sulfanilamide. The experiment was set up in triplicate with one series containing no nucleoprotein extract, a second containing extract from the resistant strain and a third with extract from the sensitive strain. Sterility controls for the extract were included and all the other tubes were inoculated with a small inoculum from a young culture of the sulfonamide resistant strain.

The results in table 1 show that the resistant culture grew slightly in the

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presence of 30 mg % sulfanilamide and that the addition to the medium of an extract of the resistant culture had no effect on this resistance. The addition of an extract from a sensitive culture, however, prevented the growth by 30 mg% and even by 20 mg% of sulfanilamide.

The experiment reported in table 2 was set up in an identical manner but was inoculated with the sensitive strain of E. coli. It is observed that this organism is completely inhibited by 5 mg% of sulfanilamide both in the control series and where an extract of the sensitive strain was added to the medium. The addition of extract from the resistant strain permitted slight growth even in 10 mg% of sulfanilamide.

A study of the quantitative changes in the population is reported in table 3. Here the organisms were grown in the presence or absence of the bacterial extracts as indicated but without any sulfanilamide added. After 24 hours the mature cultures were plated in agar containing varying amounts of sulfanilamide to determine the distribution of resistance in the resulting population. As can be seen from the plate counts the presence of an extract from a resistant culture increases the average resistance of a sensitive population. And more surprising the presence of extract from sensitive cells decreased the average resistance in the resistant population.

An experiment utilizing the purified nucleic acid extracts prepared as indicated in table 4 showed that these contained the "transforming principle".

The most obvious explanation of this conception assumes that mutations result from inexact replications of the genetic mechanism of the microbe and that these "errors" are found in the nucleic acid. Under some conditions organisms may assimilate these preformed fragments of their genetic control mechanism if they are added to the medium. If the fragments are slightly different from those ordinarily built by the organisms the result is a mutation.

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From the data presented here it must be assumed that the highly resistant organisms of the resistant population prefer to absorb the nucleic acid from the normal culture rather than duplicate their own genetic mechanism which is abnormal as compared with the main population.

Table 1.

TRANSMUTATION OF SULFONAMIDE RESISTANT

E. COLI

Sulfanilamide (mg %)	Nucleoprotein Extract: none	resistant	sensitive
0	++++	++++	++++
2	++++	++++	++++
5	++++	++++	++++
10	++++	++++	++
20	+++	+++	-
30	+	+	-

Continued

Table 2.

TRANSMUTATION OF SULFONAMIDE SENSITIVE

E. COLI

	Nucleoprotein Extract		
Sulfanilamide	None	Resisant	Sensitive
0	++++	++++	++++
1	++++	++++	++++
2	++	+++	++
3	+	+++	+
5	-	++	-
7	-	+	-
10	-	±	-

Table 3.

PLATE COUNTS OF *E. COLI* ON SYNTHETIC AGAR CONTAINING VARYING SULFANILAMIDE CONCENTRATIONS

		Mg % Sulfanilamide				
Strain	Extract	0	2	5	10	20
Sensitive	none	130 M	2 M	900	0	0
Sensitive	resistant	120 M	7 M	20 T	100	0
Resistant	none	140 M	112 M	8 M	800 T	900
Resistant	sensitive	140 M	100 M	420 T	2 T	0

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Table 4.

PREPARATION OF NUCLEIC ACID

1 gram wet cells - .1 M Na citrate in .2% Na desoxycholate.
Heat to 50° C. for 10 minutes. Centrifuge.

cell debris
discard

crude extract
+ 4 volumes of EtOH

other precipitate
and dissolved substance

fibrous precipitate
(crude nucleoprotein)
di
dissolve in M NaCl
shake with 1/10 vol
amyl alcohol and 1/3 vol
chloroform

protein

nucleic acid